AD 676952

TRANSLATION NO. 156

DATE: Sept 1968

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Fort Detrick
Frederick, Maryland

Variability and Selection of Microorganisms*

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The history of microbiology reminds us that the variability of microorganisms had already drawn the attention of researchers who created microbiology. Nevertheless, in the following years study was applied especially to the morphological aspect, descriptive of the problem.

Researchers have interested themselves very little with the valuable practical properties of microbes, which is why we have very little today on the theory of selection of microbes. Achievements in plant selection are incomparably superior in this respect. However, the greater practical value of microbes incites theoretical research on variability of microorganisms.

The specific structure of the bacterial cell is related to the problem of heredity and variability of microbes.

For sixty years the nucleus or chromosomes of the bacterial cell were described as being isolated morphological formations.

Now, these discoveries are not confirmed by the following. For the last fifteen years attention has been drawn by the works of Robinow and certain of his adopts (Bisset and others). Robinow discovers the nucleus of bacteria and describes their division. Minute verification of the data of Robinow leads us to make the following conclusions:

1. The forms which he took for nucleii are polar grains situated at the poles of the cell (fig.1). After staining by the Giemsa method the cell and the polar grains swell greatly, the latter are moved toward the center of the cell (fig 1)

2. Swelling more marked at the extremities of the cell, variation of distance existing between the enlarged polar corpuscles, all this imitates different stages of the division of nucleus and cell. (fig 2)

3. The phenomenon of swelling of the polar grains is produced when the bacteria are stained by the Giemsa method; on the other hand, proceeding by other methods of staining, this phenomenon is no longer produced. That is why the living cells or those stained by other methods have no "nucleus".

4. Consequently, the bacterial nucleus of Robinow are only an artefact.

As shown by the research of soviet biochemists, bacteria are very rich in thymonucleic acid. If this latter were concentrated in one point, the nucleus of the cell would be visible under weak magnification. In reality, the nuclear substance is diffused through the whole cell, which is why bacteria should be considered as cellular organisms with relatively primitive organisation. Their existence is one of the proofs of evolution of the cell. Bacteria being capable of inheriting their characters in the absence of chromosomes proves that the whole cell is "bearer" of hereditary character. That demonstrates besides the possibility of conserving over a long period of time the valuable practical properties of microbial cultures.

Controlled Variability

The variability of organisms in general and that of microorganisms in particular depends on their development in the conditions of inhabitual life. Organisms acquire new properties entirely corresponding to new conditions of existence. The disciples of I.I. Metchnikov have demonstrated in their works that through successive passages on culture media with growing concentrations of antiseptics, one can form races of bacteria resistant to these antiseptics. While cultivating yeasts on media containing antiseptics (cetylpyridinium bromide) N G. Verbina obtained (~~see drawings of fig. 3~~) cultures more resistant to this antiseptic than the initial form. In our laboratory L.G. Loguinova while cultivating yeasts Saccharomyces cerevisiae at progressively increasing temperatures, obtained thermophilic yeasts which ferment carbohydrates of must at 40° although the original yeasts scarcely multiply at this temperature. (~~fig. 4~~)

This adaptation to elevated temperatures has modified the type of development of the yeasts. They acquire characteristics very different from their initial form: their cells become much smaller, their biochemical activity becomes more intense, giving more alcohol per unit of weight (dry); etc.

Still, the most interesting fact from the theoretical point of view is that the adaptation of yeasts to a higher temperature modifies the tempo of their growth.

From figure 5 it is evident that these yeasts multiply more rapidly at high temperature than the original yeasts at 30°. As we established earlier, one of the particular characteristics of the thermophiles lies in the fact that they multiply more rapidly than mesophiles.

As to the thermophiles, all their processes are more intense and rapid, for they are unfolded at a higher temperature; that concerns especially the rapidity of multiplication. The process of formation of thermophiles in nature has, then, been successfully reconstructed experimentally in the laboratory and it has been demonstrated that the process is followed by the appearance in the metabolism of microbes of specific characteristics for thermophiles.

It is incontestable at present that while controlling variability, one can obtain races of microbes resistant to no matter what physical and chemical factor. Thus, in the Soviet Union there have been obtained cultures of microbes resistant to different antibiotics, cultures of yeasts standing strong concentrations of carbohydrates, alchohol, acids, etc. One can readily obtain roots of mold or of yeasts resistant to different antiseptics; these races provoke fermentation of media containing antiseptics and do not undergo from this the unfavorable action of bacteria.

However, although we easily obtain forms resistant to physical and chemical factors, we cannot yet sufficiently control the metabolism of microbes to be able to modify the whole microbial character presenting a practical interest. We know that cultivating bacteria in media with concentrations of certain vitamins, successively diminished, one can succeed in creating forms synthetizing these vitamins and no longer in need of receiving them ready-made. Even while modifying the composition of the nutritive medium we can obtain certain hydrolases produced by the yeasts; maltases, galactases etc. Nevertheless, using only incomplete data, we cannot at present strengthen, in the desired sense, the fermentive activity of microbes, their faculty of synthetizing fats, etc. Research to be undertaken in the immediate future should discover conditions which will permit activating the necessary functions. The practical range of controlled variability is immense. This method has resulted in the creation of yeasts resistant to alcohol, bacteria whose existence depends on streptomycin and which reveal the presence of this antibiotic, and numerous other races of microbes, having found application in different branches of industry. The controlled modification of hereditary characteristics of microbes is the principal method of selection of microorganisms.

On the correlation between physiological and morphological modifications

It is evident to all microbiologists that the exterior form of the colony, the character of growth on different nutritive media etc. depends on the metabolism of the microbes and constitutes their expression.

The metabolism is primary, since all modification of the morphology of microorganisms has a secondary characteristic, for morphology insofar as united to the function, can be considered only as a consequence of physiological modifications. For yeats we have studied in our laboratory the correlative variability, that is, the physiological characteristics of variants of microorganisms which morphologically differ from the initial form. It has been established in particular that the rough forms, as shown in fig. 6 of

Penicillium chrysogenum, form twice as much gluconic acid (fig.7) as the original smooth forms of champignon. The rough races of Aspergillus niger obtained experimentally give proof of very pronounced variability in fermentive ability. As fig. 8 proves, the appearance of these forms is very different. The rough forms give off amylases and proteases more active than the original smooth forms, as fig. 9 indicates.

The fermentive activity of variants increases by unit of weight (dry), in consequence, this increase could not be explained by a mass growth of cultures.

One observes a considerable difference in morphology, the metabolism of rough and smooth races of Acetobacter suboxydans (fig.10)

One can select from among the rough races of Acetobacter suboxylans those which multiply more intensely than the smooth initial forms (fig. 11) and their cultures oxydize sorbitol more than the initial smooth form. General laws have also been established for spore-bearing bacteria. In particular the typical smooth variants of Bac. mesentericus multiply better than the initial smooth forms, as shown in fig. 12. One could multiply examples of the modification of metabolism of microbes which entails changes in the morphology of colonies and cells. From considerable research we can formulate the following general conclusions:

1) Modification of the metabolism of microbes entails morphological changes. As example one can cite the creation of rough forms of microbes which contrary to the theory of dissociation, can be biochemically more active.

2) The formation of rough variants is not linked, as the microbial theory of dissociation presumes, to the passing of one phase in the evolutionary cycle of the microbe, but presents a specific case of variability.

3) Rough races formed are not identical and may have different biological properties. In particular they may have reduced biochemical activity. However, as it appears from experimental research and from the data of writers, one can detect among the rough forms races with increased biochemical activity insofar as it concerns the living mass, the facility of oxidation, formation of ferments and antibiotics etc. These forms of more intense activity have not been revealed on examining a large number of initial smooth stock.

4)Selection of valuable races for practice can also be realized on the basis of exterior morphological characteristics, which is an unquestionable interest in the selection of microorganisms. It goes without saying that after the selection, one must proceed to a larger study of the metabolism of microbes.